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A Study of Neonatal Septicaemia in a Tertiary Care Hospital

Piyush Garg[1] and M.G. Usha[2]

[1]JJM Medical College, Davanagere, Karnataka 577004, India.
[2]Department of Microbiology, JJM Medical College, Davanagere, Karnataka, India.

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Neonatal sepsis is one of the most common cause of death among neonates in the developing countries. Neonatal infections are estimated to cause 1.6 million deaths every year all over the world and 40 % of deaths occurring in the developing countries like India. To determine the bacteriological profile and antibiogram in neonatal septicemia cases. 99 different neonates, clinically diagnosed were included. Blood specimens for culture were drawn. Bacteria was identified, antibiogram was determined and ESBL test was done Out of 99 clinically suspected cases, 44 cases were culture positive whereas 55 were culture negative. Gram positive bacteria were the most common isolates (68.18%) followed by gram negative bacteria (31.82%). Among the Gram positive bacteria, MRCONS (50%) was the most common followed by MRSA (%). Among gram negative bacteria, Klebsiella (11.36%) was the most common followed by acinetobacter (9.09%), *E.coli* and *Pseudomonas*. 93.18% of the cases were EOS whereas 6.82% cases were LOS.92% of CONS were sensitive to linezolid, 84% to chloramphenicol, and 68% to amikacin. All isolates of *Staphylococcus aureus* were sensitive to linezolid and chloramphenicol. Most of the Gram negative bacteria were resistant to the most commonly used antibiotics.64.28% of Gram negative bacilli were ESBL producers of which *Klebsiella* was the commonest (44.44%). The increasing spread of different bacteria differing in resistance patterns demands for evidence based practice in neonatal septicemia.

Keywords: Neonatal Septicemia, Blood Culture, ESBL producers.

Neonatal sepsis or septicaemia is a clinical syndrome of bacteraemia characterised by signs and symptoms of infection in first month of life. It refers to generalised bacterial infection with a positive blood culture in the first four weeks of life. [1, 2] According to national neonatal perinatal database (NNPD) 2002-2003, the incidence of sepsis is reported to be 30 per 1000 live births. [3, 4] Several studies done earlier show that *Klebsiella pneumoniae* (32.5%) was the most frequent Gram negative bacteria isolated, followed by Gram positive *Staphylococcus aureus* (13.6%). [3, 5] The most common organisms responsible are multidrug resistant gram negative bacilli particularly members of family enterobacteriaceae and non-fermenting gram negative bacilli.[6] Further the emergence of extended spectrum Beta-lactamases (ESBLs) producing Gram negative bacteria have led to bad prognosis.

Although the common factors associated with these infections are low birth weight, duration of hospital stay, invasive procedures, surgery and also colonization by bacteria from hospital environment, a significant proportion of these septicemia babies are those, who were born unattended outside the hospital in unhygienic environment. The neonates suffering from sepsis presents with many symptoms such as vomiting, poor feeding, respiratory distress, fever, diarrhoea, cyanosis, tachycardia or bradycardia, lethargy, irritability, abdominal distension, hepatomegaly,

* To whom all correspondence should be addressed.
E-mail: 17piyush12@gmail.com

oliguria, pallor, cold, hypotension, seizures, hyporeflexia, hypotonia, jaundice, etc.

There are two classes of neonatal sepsis on basis of time of onset –

1. Early onset sepsis (EOS) – it is mainly due to bacteria acquired before and during the birth of the child.
2. Late onset sepsis (LOS) - it is due to bacteria acquired after birth.

Different researches distinguish between the types as- very early onset (within 24 hours of birth), EOS (24hours to six days) and LOS (more than six days)[7] While according to NNPD, early onset sepsis is within 72 hours whereas late onset sepsis is after 72 hours of birth[1, 3, 4]

Early diagnosis and proper management of neonatal septicaemia can bring down the morbidity and mortality. The bacteria causing sepsis differ in different regions and in different hospitals. Hence, the present study was done with an objective to determine the local patterns and antibiotic sensitivity of etiological agents which is very important to know the trends in infection and for better management of neonates with septicaemia.

MATERIALS AND METHODS

Type of study: Descriptive, hospital based 99 different neonates who were clinically diagnosed of having neonatal septicaemia attending todepartment of neonatology/NICU/ paediatric ICU of our hospital were included. Duration of symptoms, age of onset of symptoms and other demographic details (birth weight, age etc.) of neonates was collected.

Inclusion criteria: The neonates suffering from neonatal septicaemia of -

- Age less than 28 days, > 22 weeks of gestation and full term babies.
- Presence of three or more clinical symptoms of neonatal septicaemia.

Exclusion criteria

- Neonates having other illness like congenital anomalies, undergone surgery.

Institutional ethical committee clearance was obtained. Written consent was taken from the parents/guardian of the neonates.

Sample collection and processing

Before administering any antibiotic, 1ml peripheral venous blood was collected following aseptic precautions using surgical spirit and povidine iodine. The sample was inoculated immediately into 5ml-10ml of brain heart infusion broth with 0.025% sodium polyanetholsulfonate as anticoagulant. Inoculated broth was incubated aerobically at 37ºC for 24 hours. Then subcultures were done in Biosafety Cabinet on to Blood agar plate and Mac Conkey agar plate.

The plates were incubated at 37ºC for 18-24 hours aerobically. Bacterial colonies produced if any were studied and identified by colony morphology, gram staining, motility and the standard biochemical reactions[8] Any sample showing no growth on the first subculture was further incubated for 6 days with subcultures done on everyday till 3rd day to obtain the colonies. After that subculture was done when turbidity appears. No growth was declared after 7 days of incubation of blood samples in BHI broth[9]

Antibiotic susceptibility pattern of the isolates obtained was studied by Kirby- Bauer's disc diffusion method on Muller-Hinton Agar, matching the inoculum with 0.5 McFarland turbidity and following standard guidelines[10] After overnight incubation, the plates were examined for the bacterial growth. The diameter of zone of inhibition around each disk was measured in mm and interpreted by referring to standard chart and the organism was reported as sensitive or resistant to the antibiotic that was tested[10]

For Gram positive bacteria, the antibiotics tested were Gentamicin, cefotaxime, ceftazidime, ampicillin, chloramphenicol, amoxicillin, erythromycin, cephalexin, ciprofloxacin, amikacin, and linezolid. (Himedia Laboratories Pvt. Ltd.)

The Methicilin resistance of Staphylococcus isolates was detected using Cefoxitin (30µg) disc on Muller-Hinton Agar.

For Gram negative bacteria, the antibiotics tested were gentamicin, amoxicillin, co-trimoxazole, piperacillin, ceftriaxone, ciprofloxacin, amikacin, cefoperazone+sulbactum. (Himedia Laboratories Pvt. Ltd.)

ESBL test- ESBL production in gram negative isolates were confirmed by double disk potentiation test (phenotypic confirmatory test). Ceftazidime (30µg) disk and a ceftazidime plus clavulanic acid (30µg/10µg) disk were placed at a distance of 20mm apart on MHA swabbed with

Table 1. Percentage of EOS and LOS among culture positive cases

Onset of Sepsis	Culture Positive
EOS	41 (93.18%)
LOS	3 (6.82%)
Total	44 (100%)

the Gram negative bacteria. Similarly, it was done with cefotaxime disk (30µg) and cefotaxime plus clavulanic acid (30µg/10µg). The plates were incubated at 37ºC overnight.

The test organism was considered to produce ESBL, if the zone size around the

(i) ceftazidime + clavulanic acid increased by >5mm in comparison to the ceftazidime disk alone

Table 2. Distribution of bacteria in culture positive cases (n=44)

Bacteria	Frequency (%)	
Gram Negative	Acinetobacter	4 (9.09)
	E.Coli	3 (6.82)
	Klebsiella	5 (11.36)
	Pseudomonas	2 (4.55)
Gram Positive	MRCONS(Methicillin resistant Coagulase negative staphylococcus)	22 (50.00)
	MSCONS(Methicillin sensitive Coagulase negative staphylococcus)	3 (6.82)
	MRSA(Methicillin resistant staphylococcus Aureus)	4 (9.09)
	MSSA(Methicillin sensitive staphylococcus Aureus)	1 (2.27)
Total	44 (100%)	

Table 3. Shows the Distribution of clinically suspected cases of neonatal septicemia and culture proven cases for the various demographic features.

Parameters		Clinically suspected cases	Culture Positive (n=44)	p value
Sex	Male	60(60.61)	26(59.09)	0.783
	Female	39(39.39)	18(40.91)	
Age of neonates(in days)	1 day	75(75.76)	33(75)	0.886
	2-4	2121.21)	10(22.73)	
	5 & Above	3(3.03)	2(4.55)	
Birth Weight(in Kgs)	Normal	44(44.44)	19(43.18)	0.754
	LBW	43(43.43)	18(40.91)	
	VLBW	9(9.09)	5(11.36)	
	ELBW	3(3.03)	2(4.55)	
Type of delivery	NVD	36(36.36)	19(43.18)	0.207
	LSCS	63(63.64)	25(56.82)	
Gestational Age	Term	60(60.61)	23(52.27)	0.129
	Preterm	39(39.39)	21(47.73)	
Place of delivery	Hospital	97(97.98)	42(95.45)	0.279
	Road	1(1.01)	1(2.27)	
	Home	1(1.01)	1(2.27)	
Person who conducted delivery	Doctor	97(97.98)	42(95.45)	0.279
	None	1(1.01)	1(2.27)	
	Others	1(1.01)	1(2.27)	

where LBW-Low birth weight, VLBW-Very low birth weight, ELBW- Extremely low birth weight, NVD- Normal vaginal delivery, LSCS- Lower segment Caesarean section.

For all the parameters described above p value was found to be not significant ($p<0.05$ is significant) (Table 3).

Table 4. Antibiotic sensitivity pattern in Gram Positive isolates.

Antibiotics	CONS (n=25)		S. Aureus (n=5)	
	No. of sensitive strains	%	No. of sensitive strains	%
Cefoxitin	3	12	1	20
Amoxicillin	2	8	1	20
Cefotaxime	2	8	1	20
Cephalexin	3	12	1	20
Ceftazidime	0	0	0	0
Gentamicin	10	40	3	60
Ciprofloxacin	10	40	2	40
Amikacin	17	68	3	60
Erythromycin	5	20	1	20
Ampicillin	2	8	0	0
Linezolid	23	92	5	100
Chloramphenicol	21	84	5	100

Table 5. Antibiotic sensitivity pattern in Gram Negative isolates

Antibiotics	Acinetobacter (n=4)		E.Coli (n=3)		Klebsiella (n=5)		Pseudomonas (n=2)	
	No. of sensitive strains	%	No. of sensitive strains	%	No. of sensitive strains	%	No. of sensitive strains	%
Co-trimoxazole	3	75	3	100	2	40	1	50
Amoxicillin	1	25	0	0	0	0	1	50
Piperacillin	1	25	0	0	0	0	1	50
Ceftriaxone	1	25	0	0	0	0	1	50
Gentamicin	1	25	3	100	3	60	1	50
Ciprofloxacin	1	25	1	33.33	2	40	1	50
Amikacin	1	25	0	0	3	60	1	50
Cefoperazone/ Sulbactum	0	0	0	0	0	0	1	50

Table 6. Distribution of ESBL producers in Gram Negative isolates

Gram Negative bacilli	ESBL		Total
	Positive	Negative	
Acinetobacter	2	2	4
E.Coli	1	2	3
Klebsiella	4	1	5
Pseudomonas	2	0	2
Total	9 (64.28%)	5(35.71%)	14

(ii) cefotaxime + clavulanic acid increased by >5mm in comparison to the cefotaxime disk alone. This increase in the zone size is due to inactivation of B-lactamase by clavulanic acid.

RESULTS

(i) Out of 99 clinically suspected cases, 44 cases were positive for bacterial culture whereas 55 were culture negative. 41 cases being early onset septicemia (93.18%) and three late onset

septicemia (6.82%) (Table 1).

(ii) Gram positive bacteria were the most common isolates (68.18%) followed by gram negative bacteria (31.82%). Among the Gram positive bacteria, MRCONS (50%) was the most common isolate whereas in gram negative bacteria, Klebsiella (11.36%) was the most common. (Table 2).

(iii) In both early onset and late onset septicemia, MRCONS (45.45%) was common isolate followed by Klebsiella (11.36%) in EOS and *E.coli* in LOS (2.27%)

(iv) Table 3: Shows the Distribution of clinically suspected cases of neonatal septicemia and culture proven cases for the various demographic features.

(v) 92% of CONS were sensitive to linezolid, 84% to chloramphenicol, and 68% to amikacin. All isolates of *Staphylococcus aureus* were sensitive to linezolid and chloramphenicol (Table 4).

(vi) Most of the Gram negative bacteria were resistant to the most commonly used antibiotics (Table 5).

(vii) Out of 14 Gram Negative Organisms, 9 were ESBL (64.28%). Out of which Klebsiella was most common (44.44%) (Table 6).

DISCUSSION

For the effective treatment of neonatal sepsis, study of the bacteriological profile with their antibiotic sensitivity pattern plays an important role. In the present study, 44.44% were culture positive. A similar result of 40% culture positivity was seen in a study by Israel *et al*[6] and 46.20% was found in another study by Desai *et al*[2] However, blood culture positivitywas very low (24.88%) in the study by Mathur *et al* [11] in 1990-91. This shows that the incidence of sepsis has increased over the past 26 years.

In our study, Gram positive bacteria were 68.18% whereas 31.82% were Gram negative cases. This is in contrast to Kamble *et al* in which 13.1% cases were due to Gram positive bacteria and 86.9% cases were due to Gram negative bacteria[1] It is also in contrast to Desai *et al*, in which 28.57% were Gram-positive[2]

CONS are now increasingly being isolated from neonates. Similar observation was found in our study, the most common isolate was MRCONS (50.00%). But this is in contrast to study by Desai *et al* and Israel *et al* in which Klebsiella was the most common isolate with 67.85% and 40% respectively. Next common isolate obtained in our study was Klebsiella (11.36%), followed by MRSA (9.09%), acinetobacter (9.09%), *E.coli* (6.82%), MSCONS (6.82%).

In this study, 93.18% cases were EOS whereas 6.82% were LOS. This may due to the infection during or after the rupture of the membranes or during the passage in the birth canal or in the labour room. This is comparable to the study by Kamble *et al* in which EOS were more (60.9%) than LOS (39.1%)[1]

In our study, we also found out that 43.18% culture positive cases were normal birth weight, followed by 40.91% low birth weight, 11.36% very low birth weight and 4.55% extremely low birth weight. This is in contrast to the study done by Kamble *et al*[1] in which 56.3% were very low birth weight followed by 34.8% low birth weight, 8.7% normal birth weight.

In our study, out of 44 cases of neonatal sepsis, 42 (95.45%) were hospital deliveries and 1 case was road delivery and home delivery each (2.27%). Both the cases of delivery outside the hospital was associated with sepsis, indicating that the chances of sepsis are very high outside the hospital. The rate of sepsis in hospital infection was also high which can be explained on the basis of the most common bacteria isolated i.e. MRCONS which tells that it is a hospital acquired infection. This study was similar to Israel *et al* [6] where 90% were hospital deliveries and 10% were home deliveries.

In our study, out of 44 cases of culture proven neonatal sepsis, 42 (95.45%) were delivered by trained people like ANMs, doctors and 2 cases (2.27%) were by untrained person. These results were similar to the results of study by Israel *et al* [6] where 90% cases were by trained persons and 10% were by untrained dais.

The result of antibiotic sensitivity shows that all Gram positive bacteria isolated were sensitive to Linezolid and Chloramphenicol.

In the present study 9 (64.28%) out of 14 Gram Negative bacilli were ESBL producers whereas in study by Kamble *et al*[1] 9 (45%) out of

20 gram negative bacilliwere ESBL producers. In our study, out of 5 isolates of Klebsiella, 4 (80%) were ESBL producers.

CONCLUSION

Sepsis is one of the most common cause of morbidity and mortality of neonates in the developing countries. The increasing spread of different bacteria differing in resistance patterns urges to submit blood samples for culure work before treatment. Earlier the microorganisms were sensitive to nontoxic penicillin and its congeners. But the increasing and indiscriminate use of antibiotics lead to the emergence of strains resistant to these as well as routinely used antibiotics like ampicillin, netilmicin, 1st and 2nd generation cephalosporin.So, the development of highly potent antibiotics like 3rd and 4th generation cephalosporins have controlled the present day scenario but the increasing and irrational use of these antimicrobial drugs will lead to resistance to these also. Hence, there is an utmost need to revise the regimen given for a particular sepsis case as the susceptibility of organisms keep on changing. So that an appropriate and rational antibiotic drug can be given for that particular organism isolated in the blood culture. The drug should be prescribed in an appropriate dose for an appropriate time to kill all the organisms in the body, so as to decrease the emergence of resistant strains and limit their spread.

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